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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/364,847	07/30/1999	OLIVER P. PEOPLES	MBX030	9982

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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Offic Action Summary	Applicati n No. 09/364,847	Applicant(s) PEOPLES ET AL.	
	Examiner David J. Steadman	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 July 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disp sition of Claims

4)☒ Claim(s) *1-6* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Application Status

Claims 1-6 are pending in the application.

Amendment to the specification and claims 1, 2, and 4 in Paper No. 23, filed 07/09/02 is acknowledged.

Applicants' arguments presented in Paper No. 23 have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

It is noted that the IDS references have been submitted by applicants for consideration by the examiner on 04/17/02. However, as of the writing of this Office action, the references have not been received by the examiner. As stated in a previous Office action, in order to ensure delivery of IDS references to the examiner, particularly a large number of references as is the instant case, it is suggested that applicants have the references hand delivered to the Group and have the receptionist contact the examiner upon arrival. The references will be fully considered upon receipt and Form PTO-1449 will be returned in a subsequent communication. It is noted that the references appear to have been mailed and not hand delivered as suggested by the examiner.

Claim Objections

1. Claims 1 and 6 are objected to because of the following informalities: the terms "beta-hydroxyacyl-ACP::coenzyme-A transferase" in claim 1 and "a bacteria" in claim 6 are grammatically incorrect and should be replaced with, for example, "beta-hydroxyacyl-ACP::coenzyme-A transferases" (emphasis added) and "a bacterium" or "bacteria". Appropriate correction is required.

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1-6 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 (claims 2-6 dependent therefrom) is directed to a fusion protein having a formula of E1-Ln-E2 or E2-Ln-E1, wherein E1 and E2 catalyze successive steps in a PHA biosynthetic pathway and are selected from a genus of beta-ketothiolases, a genus of acyl-CoA reductases, a genus of PHA synthases, a genus of PHB synthases, a genus of phasins, a genus of enoyl-CoA hydratases, and a genus of beta-hydroxyacyl-ACP::coenzyme-A transferases which have not been adequately described in the specification. Claim 2 limits E1 and E2 of the fusion protein of claim 1 to a genus of phaAs and phaBs, a genus of phaCs and phaPs, a genus of phaCs and phaGs, and a genus of phaCs and phaJs. The specification teaches the structures of only six representative species of such fusion proteins, i.e., SEQ ID NOs:10, 19, 33, 35, 49, and 51. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a fusion protein of the individual constituent enzymes as recited in claims 1 and 2. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

3. Claims 1-6 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a fusion protein selected from the group consisting of *Alcaligenes eutrophus*, *Pseudomonas oleovorans*, *Zoogloea ramigera*, and *Aeromonas caviae* phbA-phbB; phbB-phbA; phaC-phaP; phaP-phaC; phaC-phbG; phbG-phaC; phaC-phaJ; and phaJ-phaC, having a peptide linker of from

zero to about 10 amino acids and wherein the expression of the fusion protein is under the control of a single promoter, does not reasonably provide enablement for *any* fusion protein of the formula E1-Ln-E2, wherein E1 and E2 catalyze successive reactions in a PHA pathway and are selected from the group consisting of: *all* beta-ketothiolases, *all* acyl-CoA reductases, *all* PHA synthases, *all* PHB synthases, *all* phasins, *all* enoyl-CoA hydratases, and *all* beta-hydroxyacyl-ACP::coenzyme-A transferases, wherein the linker is *any* length, and the expression is under control of a single promoter (claim 1), and optionally wherein E1 and E2 are selected from *any* phaA and phaB; *any* phaB and phaA; *any* phaC and phaP; *any* phaP and phaC; *any* phaC and phaG; *any* phaG and phaC; *any* phaC and phaJ; and *any* phaJ and phaC (claim 2), and optionally wherein the linker is up to 50 amino acids (claim 3). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 1 (claims 4-6 dependent therefrom), 2 and 3 are so broad as to encompass *all* fusion proteins as described above. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of constituent enzymes and linkers broadly encompassed by the claims.

The claims are so broad as to encompass constituent enzymes from *any* source. While techniques of gene isolation are well-known in the art, the art recognizes that these techniques, e.g., hybridization and PCR, are highly dependent upon the structural homology of the hybridizing strand or primers used in the methods. Therefore, the genes encoding the constituent enzymes as disclosed in the specification from *Alcaligenes eutrophus*, *Pseudomonas oleovorans*, *Zoogloea ramigera*, and *Aeromonas*

caviae may not be useful for the isolation of genes encoding *all* constituent enzymes as broadly claimed. Furthermore, the specification and the prior art provide no guidance as to which of the infinite number of sources, i.e., organisms, are likely to comprise such genes. Also, neither the specification nor the prior art provides guidance as to which of the infinite number of combinations of constituent enzymes will be useful as not all products of one constituent enzyme will be substrates for a second constituent enzyme and one of skill in the art would be required to screen for compatible enzyme pairs for a fusion protein. Therefore, there exists a high degree of uncertainty as to whether the disclosed genes encoding the constituent enzymes can be used to isolate *all* nucleic acids encoding said constituent enzymes for the generation of the claimed fusion enzyme.

Also, The peptide linker of claim 1 (claims 2 and 4-6) has any number of amino acids and the peptide linker of claim 3 has from zero to fifty amino acids. Applicants have provided guidance and working examples of the fusion proteins of SEQ ID NOs:10, 19, 33, 35, 49, and 51, having only two amino acids linking the two individual enzyme sequences. The prior art teaches that linkers from two to ten amino acids are optimal and that longer linkers are often prone to proteolytic degradation (page 230 of Bulow et al. Trends Biotechnol 9:226-231). Therefore, there is a high degree of unpredictability in making the claimed fusion proteins with a linker of any number of amino acids or greater than 10 amino acids in length.

The specification does not support the broad scope of the claims which encompass *fusion* enzymes as described above because the specification does not establish: (A) methods for isolating genes encoding all constituent enzymes as broadly encompassed by the claims; (B) guidance as to the presence of these genes in other organisms; (C) guidance as to using linkers of greater than ten amino acids with a reasonable predictability of no proteolytic degradation of the linker; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims

broadly including *all* fusion enzymes as described above. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

4. In view of the Declaration submitted under 37 CFR 1.131, the rejection of claims 1-3, 5, and 6 under 35 USC 102(e) as being anticipated by Srienc (US Patent 6,143,952), is withdrawn.

Claim Rejections - 35 USC § 103

5. In view of the Declaration submitted under 37 CFR 1.131, the rejection of claim 4 under 35 USC 103(a) as being unpatentable over Srienc in view of Bulow and Argos, is withdrawn.

6. Claims 1-3, 5, and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Peoples et al. (IDS reference; US Patent 5,245,023; hereafter referred to as "Peoples") in view of Bulow et al. (Trends Biotechnol 9:226-231; hereafter referred to as "Bulow"). Claim 1 is drawn to a protein fusion consisting of enzymes that catalyze successive reactions of the PHA biosynthetic pathway as recited in the claim separated by a linker peptide and wherein expression is under the control of a single promoter. Claim 2 limits the enzymes of the fusion protein of claim 1. Claim 3 limits the linker of the fusion protein of claim 1 to between zero and fifty amino acids. Claims 5 and 6 limit the fusion protein to being expressed in a plant or bacteria, respectively.

Peoples teaches the potential utilities of PHB as a commercially useful complex biopolymer (column 1). Peoples teaches that three enzymes are involved in the synthesis of PHB – beta-ketothiolase, acetylacetyl-CoA reductase, PHB polymerase (column 1). Peoples teaches the isolation of genes encoding beta-ketothiolase, acetylacetyl-CoA reductase, PHB polymerase and PHA polymerase from *Zoogloea ramigera* and *Alcaligenes eutrophus* (columns 6-14). Peoples teaches "[b]y combining these enzymes in

either bacterial or plant cells... ...a variety of polymers can be constructed" (columns 4 and 5). Peoples teaches co-expression of the beta-ketothiolase, acetylacetyl-CoA reductase, and PHB polymerase genes in *Escherichia coli* results in the formation of PHB (columns 17-19). Peoples teaches the beta-ketothiolase, acetylacetyl-CoA reductase, PHB polymerase and/or PHA polymerase can be co-expressed in plants and teaches methods of engineering plant cells for expression thereof for the production of PHB (columns 26 and 27). Peoples teaches a specific example of a PHB synthase-PHA synthase fusion for production of PHA polymers by stating, "the cloned and characterized [PHB and PHA polymerase] genes can be further modified by constructing fusions of the polymerases" (column 23). It is noted that the *Pseudomonas oleovorans* PHA synthase 1 (or phaC1) of SEQ ID NO:21 is also referred to as PHA polymerase in the sequence listing paper copy at page 17. It is further noted that the *Zoogloea ramigera* PHB synthase (or phaC) of SEQ ID NO:36 (encoding SEQ ID NO:37) is referred to as phbC in the sequence listing paper copy at page 33. Peoples refers to their PHB polymerase as being encoded by a phbC gene (column 4). Therefore, it appears that PHA polymerase is synonymous with PHA synthase and PHB polymerase is synonymous with PHB synthase. Peoples does not teach the limitation of a linker peptide linking the PHB polymerase and the PHA polymerase or the limitation of expression of the fusion protein being under the control of a single promoter.

Fusion enzymes were well-known to an ordinarily skilled artisan at the time of the invention. As a representative example, Bulow reviews the current (as of 1991) state of the art regarding multi-enzyme systems obtained by gene fusion. Bulow teaches a bi-functional enzyme can be prepared by joining the genes of two enzymes by removing the translational stop signal at the 3'-end of the first gene and ligating the ATG-start codon of the second gene in-frame with the first gene (page 227, left column). Bulow continues by stating that the enzyme selected to be at the N-terminal end is arbitrary and that the native tertiary structure of the fused enzymes remains almost intact (page 227, left column) and that if the entire primary sequences of the fused native enzymes are maintained in the fusion, the enzymes usually retain most of their native specific activities despite being fused together (page 230). Bulow teaches the presence of a linker peptide of two to ten amino acids is used to separate the fused native

enzymes (page 230). Bulow teaches that the use of hybrid bi-functional enzymes in biotechnology offers many advantages (page 231). Bulow teaches artificial bi- and poly-functional enzymes will be most useful in the development of metabolic engineering (page 231). Bulow teaches that such artificial enzymes will be most beneficial in directing a substrate to a preferred metabolic route (page 231). Bulow teaches that due to proximity effects, an intermediate product can be transferred efficiently to a desired second enzyme, instead of a competing enzyme (page 231). Bulow teaches the preparation of such artificial multi-enzyme systems will probably be most valuable in the construction of industrial microorganisms (page 231).

Neither Peoples nor Bulow teaches or suggests the limitation of expression of a fusion enzyme be under the control of a single promoter. However, one of ordinary skill in the art at the time of the invention would have used a single promoter for expression of a fusion enzyme.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Peoples and Bulow for a fusion protein catalyzing successive reactions of the PHA biosynthetic pathway such as a beta-ketothiolase-acetylacetyl-CoA reductase fusion enzyme, an acetylacetyl-CoA reductase-PHB polymerase or -PHA polymerase fusion enzyme, or a PHB polymerase-PHA polymerase fusion enzyme having a linker of between two and ten amino acids and expressed by a single promoter. One would have been motivated to make a fusion enzyme catalyzing successive steps of the PHA biosynthetic pathway because of the teaching of Peoples who suggests a PHB polymerase-PHA polymerase fusion as described above and the combined teachings of Peoples who teaches co-expression of beta-ketothiolase, acetylacetyl-CoA reductase, PHB polymerase or PHA polymerase genes results in the formation of PHB and Bulow who teaches advantages, e.g., proximity effects and metabolic flux, of using fusion enzymes in metabolic engineering for increased production of PHB. One would have been motivated for a linker of between two and ten amino acids linking the individual enzymes because of the teachings of Bulow as described above. One would have a reasonable expectation of success for a fusion protein catalyzing successive reactions of the PHA biosynthetic pathway such as a beta-ketothiolase-acetylacetyl-CoA reductase fusion enzyme, an acetylacetyl-CoA reductase-PHB polymerase or -PHA polymerase fusion

enzyme, or a PHB polymerase-PHA polymerase fusion enzyme having a linker of between two and ten amino acids and expressed by a single promoter because of the teachings of Peoples who teaches the nucleic and amino acid sequences of beta-ketothiolase, acetylacetyl-CoA reductase, PHB polymerase, and PHA polymerase and/or methods of isolation thereof and Bulow who teaches preparation of a fusion enzyme. Therefore, claims 1-3, 5, and 6, drawn to a protein fusion consisting of enzymes that catalyze successive reactions of the PHA biosynthetic pathway as recited in claim 1 separated by a linker peptide and wherein expression is under the control of a single promoter would have been obvious to one of ordinary skill in the art.

It is noted that the examiner has applied the common knowledge of a single promoter driving the expression of a fusion protein. The cited common knowledge is capable of instant and unquestionable demonstration as being well known in the art. MPEP 2144.03 states, "If justified, the examiner should not be obliged to spend time to produce documentary proof" (see *In re* Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942)). If applicants should challenge a factual assertion presented by the examiner as not properly based upon common knowledge, applicants should specifically point out the supposed errors in the examiner's Office action, including stating why the noticed fact is not considered to be common knowledge or well-known in the art. See MPEP 2144.03 and 37 CFR 1.111(b) regarding reliance on common knowledge in the art.

Applicants' traversal of the previous rejection under 103(a) as it applies to the reference of Bulow is addressed below.

7. Claim 4 is rejected under 35 U.S.C. 103(a) as being unpatentable over Peoples in view of Bulow as applied to claims 1-3, 5, and 6 above, and further in view of Argos (J Mol Biol 211:943-958, 1990). Claim 4 is drawn to the protein fusion of claim 1 with a linker comprising glycine and serine.

Peoples and Bulow disclose the teachings as described above. Neither Peoples nor Bulow teach the limitation of a linker comprising glycine-serine.

Argos teaches advantages of using an oligopeptide linker comprising glycine, serine, and threonine (page 947). Such advantages include flexibility of the linker provided by glycine due to its

relatively small side chain, conformational and energetic stability due to hydrogen bonding of the polar side chain of serine and threonine with solvent in an aqueous environment and reduced susceptibility to of an oligopeptide linker comprising glycine, serine, and threonine to proteolysis (page 947).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Peoples, Bulow, and Argos for a fusion protein catalyzing successive reactions of the PHA biosynthetic pathway such as a beta-ketothiolase-acetylacetyl-CoA reductase fusion enzyme, an acetylacetyl-CoA reductase-PHB polymerase or -PHA polymerase fusion enzyme, or a PHB polymerase-PHA polymerase fusion enzyme having a linker of between two and ten amino acids comprising glycine, serine, and threonine and expressed by a single promoter. One would have been motivated to make a fusion enzyme as described above having a linker comprising glycine, serine, and threonine because of the advantages of using such a linker as described by Argos. One would have a reasonable expectation of success for a fusion protein as described above having a linker comprising glycine, serine, and threonine because of the teachings of Argos as described above. Therefore, claim 4, drawn to the protein fusion of claim 1 having a linker comprising glycine and serine would have been obvious to one of ordinary skill in the art.

Applicants argue that a prophetic reference does not provide for an enabling disclosure. Applicants argue the reference of Argos fails to teach a genetic linker that would not hinder the expression of the two fused active enzymes and Argos fails to teach a single promoter for expression of the fusion protein. Applicants' argument is not found persuasive. Argos provides a rigorous analysis of linker peptides and provides clear guidelines for such a linker in terms of linker size and composition. Bulow teaches the use of linkers for fusion of constituent enzymes, provides guidance on linker length, and suggests that such fusion proteins would be catalytically active, by stating for example, that "[i]f the entire primary sequences of the native enzymes are maintained in the fusion enzymes, the enzymes usually retain most of their native specific activities, despite being fused together" (page 230). As demonstrated by the reference of Bulow, the state of art at the time of the invention therefore suggests that such fusion proteins are catalytically active. Furthermore, as demonstrated by the example of Bulow, fusion enzymes and the preparation thereof were well-known to one of ordinary skill in the art at the time

of the invention. Although Peoples provides a prophetic example of a fusion protein (PHB polymerase-PHA polymerase fusion enzyme), one of ordinary skill in the art, based on the teachings of Argos and Bulow, would have had a reasonable expectation of success for making a catalytically active fusion protein as encompassed by the claims.

Conclusion

8. No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Thursday from 6:30 am to 5:00 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Group is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600